REMARKS

Claims 89-95, 101-127 are pending in the application for the Examiner's review. Claims 96-98, and 100 have been canceled in the present amendment without prejudice. Applicants reserve the rights to prosecute canceled subject matter in one or more related applications. Claims 89, 90, 93, 94, 95, 105, 108, 112, 113, 114, 115, 121, 122, 123, 124, and 126 have been amended to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The amendments are supported in the specification and the originally filed claims. No new matter has been added. Entry of the following remarks to the file are respectfully requested.

I. THE REJECTIONS UNDER 35 U.S.C. § 112

A. The Rejection Under 35 U.S.C. § 112, Second Paragraph, Is In Error

Claims 89-98 and 100-127 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner alleged that the phrase "derived from" in claims 89 and 105 is vague and renders the claims indefinite. Claims 96-98, and 100 have been canceled. Accordingly, rejection to these claims are rendered moot. Although Applicants do not agree with the Examiner, in order to expedite prosecution of this application, claims 89, 105, and 124 have been amended by replacing the phrase "derived from" with "obtained from". Support for this amendment may be found, *inter alia*, on page 15, lines 9-11; page 20, lines 19-22, and 31-34; page 26, lines 14-16 of the originally filed specification.

The Examiner on page 12 of the Office Action states that it is unclear as to the metes and bounds of what would be considered "derived from", and that the phrase "flanking sequences that are derived from said inbred strain of animal" does not mean that the flanking sequences are 100% identical to the corresponding sequence in the target DNA sequence of genome. As discussed above, the claims have been amended to recite "obtained from" to show that the flanking sequences are obtained from the strain of inbred mouse as the corresponding sequence in the target DNA.

The Examiner also alleged that it is unclear whether "said inbred strain of animal" in lines 10-11 of claim 89 refers to "an inbred strain of animal" in line 2 of claim 89, "an inbred strain of an animal" in lines 2-3, or "an inbred strain of animal" in line 6. Applicants have amended claim 89 to provide proper antecedent basis for "inbred strain of mice". Thus, the phrase "inbred strain of mice" has a consistent meaning throughout claim 89. Claims 115, 121,

122, 123, and 124, which are dependent from claim 89, have also been amended to recite "inbred strain of mice". Support for this amendment may be found, *inter alia*, on page 27, line 31 to page 29, line 1 of the originally filed specification.

The Examiner alleged that claim 112 recites the phrase "said introduction step comprises . . ." which is vague and renders the claim indefinite. The Examiner alleged that it is unclear what other introduction step is intended other than the steps recited in the claim. Although Applicants do not agree with the Examiner, in order to expedite prosecution of this application, claim 112 has been amended by replacing the phrase "said introduction step comprises the use of" with "said introduction step uses". As such, the amended claim 112 is clear and definite.

The Examiner alleged that claim 108 recites the phrase "and/or a termination signal" which is vague and renders the claim indefinite. Although Applicants do not agree with the Examiner, in order to expedite prosecution of this application, claim 108 has been amended to recite, in part, a composition wherein said gene comprises a transcriptional start signal, a translational start signal, a termination signal, or a combination thereof. Support for this amendment may be found, inter alia, on page 9, lines 1 to 33 of the originally filed specification.

Accordingly, claims 89-95 and 101-127 are definite. Withdrawal of rejections of these claims under 35 U.S.C. § 112, second paragraph, is respectfully requested.

B. The Rejection Under 35 U.S.C. § 112, First Paragraph, Is In Error

Claims 89-98 and 100-127 have been rejected under 35 U.S.C. § 112, first paragraph. The Examiner alleged that the specification, while being enabling for a composition comprising modified embryonic stem (ES) cells of an inbred strain of mouse, does not reasonably provide enablement for a composition comprising various other modified cells of an inbred strain of animal.

Although Applicants do not agree with the Examiner, in order to expedite prosecution of this application, claim 89 has been amended to recite an in vitro composition of cultured embryonic stem cells comprising modified embryonic stem cells of an inbred strain of mice and one or more of: (i) unmodified embryonic stem cells of an inbred strain of mice; (ii) progenies of the modified embryonic stem cells; and (iii) progenies of the unmodified embryonic stem cells, said modified embryonic stem cells each comprising at least one modification sequence. Claims 90, 93, 94, 95, 113, 114, 122, 124, and 126, which are dependent on claim 89 have also been amended to recite an in vitro composition of embryonic stem cells comprising modified embryonic stem cells of an inbred strain of mice. As such, the

rejection to claims 89-98 and 101-127 have been obviated. Withdrawal of the rejection of these claims under 35 U.S.C. § 112, first paragraph, is respectfully requested.

II. THE REJECTIONS UNDER 35 U.S.C. §§ 102 AND 103

The Examiner maintains the rejections largely on the basis of the disclosures in two references by Mario R. Capecchi. The rejections appear to hinge on the interpretation of the meaning of the terms "homology" or "extent of homology". Applicants believe that the Examiner's interpretation are inconsistent with the usage of the term by Capecchi in his publications, and thus the rejections are erroneous.

Reeck (1987, Cell 50:667) submitted by Applicants, criticizes the imprecise use of the term "homology" in scientific literature at the time. According to Reeck, the term "homology" has the precise meaning of "having a common evolutionary origin", but it also carries "the loose meaning of possessing similarity or being matched" because of its improper rampant use to descibe protein and nucleic acid sequence comparisons (p. 667, left column, first paragraph).

In the rejections, the Examiner insists on applying the latter improper meaning when interpreting Capecchi's use of the term in his publications. For the reasons described below, Applicants submit that Capecchi uses the term properly and consistently to convey the **evolutionary relationship** between targeting DNA and target DNA. The term is not used by Capecchi to discuss **nucleic acid sequence comparisons** between targeting DNA and target DNA.

The following references by Capecchi are cited in the rejections:

(i) Capecchi, 1989, Trends in Genetics, 5(3):70-76 ("Capecchi C06"); and

(ii) U.S. Patent 5,464,764 issued to Capecchi et al. (" '764 patent").

Applicants have previously submitted two other references by Capecchi and his group:

(iii) Capecchi 1989, Science 244:1288-1292 ("Capecchi 1989") which contain the data disclosed in the '764 patent; and

(iv) Thomas and Capecchi 1987, Cell 51:503-512 ("Thomas", submitted herewith in the Supplemental Information Disclosure Statement and List of References Cited by Applicant as C76) which is identified as reference #31 in Capecchi C06 and as reference #8 in Capecchi 1989.

A. The Rejections Based On Capecchi C06 Are In Error

Claims 89-98, 100-127 have been rejected under 35 U.S.C.§ 102(b) as being anticipated by Capecchi C06 or in the alternative, under U.S.C.§ 103(a) as obvious over

Capecchi C06.

The Examiner alleged that the term "homology" **implies** similarity between sequences and encompasses 100% identity (page 11 of the Office Action, lines 17-20) on the basis of Reeck's observation that the term has a looser meaning of "possessing similarity or being matched" in certain scientific literature. Applicants submit that the Examiner has not provided any other basis to support the **implication** that Capecchi uses the term loosely to mean "nucleotide sequence identity."

The Examiner further alleges that one of ordinary skill in the art would envision that the higher similarity (including 100% identity) between two DNA sequences would lead to a higher frequency of recombination between these two DNA sequences, after reading in Capecchi C06 that

> the frequency of recombination between co-introduced DNA molecules is roughly proportional to the extent of homology between them. When DNA molecules share more than 5 kilobases of homology, then nearly every molecule introduced into the cell nucleus participates in at least one recombination event.

Applicants disagree because, as pointed out in applicants' Submission Under 37 CFR § 1.114 filed on November 27, 2007 ("Submission"), Capecchi C06 is referring to the **length** of the homologous regions rather than to the degree of sequence identity. There are at least two instances in Capecchi C06 that clearly show that the term "homology" does not mean nucleotide sequence similarity and is used properly in the context as Reeck recommended.

The first instance, discussed in applicants' Submission, was in the two sentences that follow immediately the mention of "extent of homology" in Capecchi C06 on page 71, left column, lines 5-11:

> When DNA molecules share more than 5 **kilobases** of homology, then nearly every molecule introduced into the cell nucleus participates in at least one recombination event. Recombination between co-introduced DNA molecules can, however, be detected between molecules sharing as little as 25-50 **base pairs** of homology. (emphasis added)

It is clear that, when Capecchi discusses the effect of the "extent of homology" on recombination frequency, he uses the unit "kilobase" and "base pairs" of homology. These

units measure the length of nucleic acids in the targeting DNA and target DNA that are of a common evolutionary origin, i.e., of mouse origin, in contrast to the vector sequence in the targeting DNA that is of bacterial origin.

The second instance appears under the section titled "TARGETED DISRUPTION OF THE *hprt* GENE" on page 72, right column, fourth full paragraph, lines 7-11.

> Futhermore, both vectors showed the same dependency of the targeting frequency on the extent of homology between the exogenous and endogenous DNA sequences. A twofold increase in homology resulted in a 20-fold increase in the targeting frequency.

Here, Capecchi is again discussing the extent of homology and its effect on targeting frequency, and mentions that a **twofold** increase in homology improved targeting frequency. One of ordinary skill in the art would understand that the twofold increase in homology represents the length of exogenous hprt sequence which was controlled in the experiment. Given the context of the experiment, the ordinarily skilled person would not interpret the sentence to mean that the nucleotide sequence similarity is increased by twofold.

The targeted disruption experiment is described in details in reference 31 of Capecchi C06 (Thomas and Capecchi, 1987, Cell 51:503-512; "Thomas") which was mentioned in applicants' Submission. Applicants respectfully point out that the statements regarding "homology" and "recombination frequency" in Capecchi C06, as well as, the data and figures disclosed in Capecci 1989 and in the '764 Patent are all based on the results in Thomas which is described in details below.

Thomas describes an experiment in which targeting vectors containing different lengths of Hprt DNA were used to disrupt an endogenous Hprt gene. According to Thomas, the Hprt gene encompasses 33 kb and contains 9 exons. The eighth exon in a cloned fragment of Hprt is disrupted by inserting a neo^r gene. The target Hprt DNA is in the genome of C57Bl/6 mouse ES cells, whereas the targeting Hprt DNA is obtained from the mouse ARK line (see p.511, first column, second paragraph, first sentence, and fifth paragraph, first sentence). Homologous recombination transfers this disruption in the targeting DNA into the endogenous Hprt gene in ES cells. (page 504, right column, first paragraph). Applicants emphasizes that DNA from two **different** mouse strains are involved in this recombination experiment.

On page 506, Figure 4 of Thomas shows the construction of three replacement targeting vectors and two insertion targeting vectors, each of which comprises the same neo^r-disrupted exon 8, and the flanking genomic sequences including exon 7 and exon 9, and different lengths

of Hprt sequences in the 5' end of the gene, i.e., 4 kb (pRV4.0), 5.4 kb (pRV5.4), 9.1 kb (pRV9.1), 3.7 kb (pIV3.7), 9.3 kb (pIV9.3). The targeting frequencies of these vectors are listed in Table 2 (page 507) and Table 3 (page 508). The results clearly showed that the frequency increases when a longer length of Hprt gene is included in the targeting DNA. In particular, Table 2 shows that the frequency obtained with the replacement vector pRV9.1 is 1/950 which is roughly 20-fold higher than the frequency of pRV4.0 which is 1/21,500 in experiment 2. The length of the portion of Hprt gene in pRV9.1 is roughly two times longer than the length of the portion in pRV4.0 (described as a "twofold increase in homology" in Capecchi C06 as discussed *supra*). A similar correlation was observed in the two insertion vectors, wherein pIV9.3 has roughly two times longer Hprt gene sequence than pIV3.7 and the frequency was increased from 1/19,000 (pIV3.7) to 1/1,100 (pIV9.3). Applicants emphasize that there is no mention of the degree of nucleotide sequence similarity between the Hprt gene in the targeting DNA in the vectors and the target DNA in the ES cells. In the Discussion section, Thomas stated that :

> The gene-targeting frequency was observed to be very sensitive to the **extent of homology** between the exogenous and cognate endogenous sequence. A 2-fold increase in **homology** increased the gene-targeting frequency by 20-fold. Further increase in the **extent of homology** may increase the gene targeting frequency even more. (on page 510, paragraph bridging the left and right columns)(emphasis added)

> Both classes exhibit comparable gene-targeting frequencies and are equally sensitive to the **extent of homology** with the endogenous target. (page 510, right column, lines 6-9)(emphasis added)

The experiments and the conclusion in Thomas indisputably show that the terms "homology" or "extent of homology" as used in Thomas as well as Capecchi C06 refer to the **length** of endogenous and targeting Hprt sequences which are of mouse origin, and not to the degree of nucleotide sequence similarity as alleged by the Examiner. Applicants submit that the ordinarily skilled person would not read Capecchi C06 or Thomas, and conclude that a higher degree of similarity between two DNA sequences would lead to a higher frequency of recombination. Capecchi merely teaches that the longer stretches of flanking DNA that is obtained from mouse in the targeting vector, the better the recombination frequency. There is nothing in Capecchi C06 that teaches using DNA from the same mouse strain in the targeting

DNA and the target DNA. Therefore, Capecchi C06 does not anticipate the claimed invention.

With respect to the rejection under 35 USC 103, the Examiner alleges that "In the event that those features are not anticipated by Capecchi, it would be obvious to one of ordinary skill in the art at the time of the invention in order to optimize the frequency of homologous recombination between the targeting DNA sequence and the target DNA sequence in the genome, ..." (page 12, last sentence) Applicants disagree with the obviousness rejection because there is no suggestion in Capecchi C06 that the recombination frequency could be increased further if DNA from the same inbred mouse strain are used in the targeting DNA and the target DNA.

Capecchi describes, in addition to the importance of the length of homologous DNA, some other observations about recombination frequency (page 71, last paragraph to page 72, first paragraph): (1) higher than anticipated recombination frequency; (2) increase in the copy number of targeting DNA and target DNA does not increase the frequency; (3) position of target gene in genome had little influence on frequency; (4) input DNA could either transfer or mutate the target DNA. None of these relate to or suggest using DNA from the same mouse strains in the targeting and target DNA.

Moreover, Applicants point out that one of ordinary skill in the art at the time would more likely optimize the experimental design according to Thomas which described the parameters that contributed to the success of the targeted disruption of the Hprt gene (page 510, fourth paragraph):

> The paramaters that we believe influenced the success of these experiments include using a neo^r gene that is efficiently expressed in ES cells, maintaining the size of the neo^r gene at a minimum, using **extensive homology** between the homing sequence and the target sequence, and removing, prior to transfection, unnecessary and nonhomologous sequences from the input vector.

Here, "extensive homology" refers to the length of homologous DNA in the targeting vector as shown to be important by the experiments. Applicants submit that at the time there was no hint anywhere in the references that the mouse strains used to make the target DNA and targeting DNA would have an impact on recombination frequency. The Examiner indicated, the strains of 129 and BALB/c mouse were well known at the time (page 12, last sentence). Yet no one at the time of the invention practice homologous recombination as described in the claimed invention, because one of ordinary skill did not believe or expect that it is necessary to use DNA from the same inbred mouse strain in the targeting DNA and the target DNA. There is

nothing in the references that would lead one to predict that the recombination frequency would be so improved if DNA from the same inbred mouse strain is used in both the vector and the target. The ordinarily skilled person would not find a reasonable expectation of success in achieving the recombination frequency of the claimed invention from the disclosure of Capecchi C06. According to case law, both the suggestion of the claimed invention and the expectation of success must be in the prior art, not in the disclosure of the claimed invention. *In re Dow Chemical Co.*, 5 U.S.P.Q. 2d 1529 (Fed. Cir. 1988). Thus, Applicant submits that any rejection of the instant claims under § 103 based on Capecchi C06 would indicate the improper use of hindsight gained from Applicants' own specification. Without the benefit of hindsight, the teachings in the reference could not possibly render obvious the claimed invention.

As discussed in the Submission and in the Declaration of the inventor, Dr. Anton Berns, dated March 14, 1995 ("the Declaration"), submitted in the Amendment dated August 2, 2007, scientists in the field of gene targeting at the time of the invention were using targeting DNA construct which was derived from a mouse strain that was different from the targeted cells. The scientists generally expected that increasing the length of the homologous region would increase the efficiency of gene targeting. The increase in efficiency was not believed to be related to the degree of sequence identity between the flanking sequence of the targeting DNA construct and the targeted cells (see paragraph 8 of the Declaration). Only after disclosure by the inventors of the present invention, for example, in Riele et al., 1992, Proc. Natl. Acad. Sci. USA 89:5128-5132 ("Riele"), did the practice of the scientists in the field started to change (see paragraph 7 of the Declaration). A copy of Riele et al. was enclosed in the Submission.

In view of the foregoing, Applicants request the withdrawal of the rejections under section 102 and 103.

B. The Rejection Based on U.S. patent 5,464,764 Is In Error

Claims 89-98, 100-120, and 122-127 have been rejected as being anticipated by U.S. Patent 5,464,764 issued to Capecchi et al. (" the '764 Patent"). Applicants submit that the '764 Patent fails to disclose each and every element of independent claim 89 and its dependent claims 90-98 and 100-127. The present invention is not anticipated because the '764 patent does not teach the use of a single inbred strain of animal as the sources of both the flanking sequences of the targeting DNA construct and the targeted cells in homologous recombination.

The Examiner is maintaining the rejection on the basis that the term "homology" used in the '764 patent should be read as "identity", despite admitting that the '764 Patent does not specifically teach 100% identity between the targeting DNA flanking sequence and the target

DNA sequence in the genome of the cells. To support this allegedly implied meaning of "homology", the Examiner relies on the looser usage of "homology" in literature on biological sequence comparisons, that is criticized in Reeck. The Examiner repeatedly alleges that the phrase "100% sequence homology" implies 100% sequence identity (page 16, lines 6-7 of the Office Action; page 17, lines 6 and 12; and page 18, lines 14-15). As discussed above, the term "homology" has been used consistently by Dr. Mario Capecchi both in Capecchi C06 and Thomas to mean "a common evolutionary origin" and not the meaning "sequence identity" that the Examiner is presently asserting. Here, the inventors of the '764 Patent are Dr. Mario Capecchi and Kirk Thomas (who is the co-author of Dr. Capecchi in Thomas). Applicants submit that the inventors also used the term "homology" in '764 Patent to mean "a common evolutionary origin" and not sequence identity.

The context in which the term "homology" is used in the '764 patent has been discussed previously in the Submission. The analysis is revisited briefly below along with Applicants' responses to the Examiner's contentions.

The term "homology" is used in the '764 Patent to convey the evolutionary relationship between targeting DNA and target DNA. In this context, 100% homology means that the targeting DNA are from the same animal **species** as the target DNA. The most illustrative use of the term "100% sequence homology" in the '764 Patent appears in column 21, lines 9-14 wherein it is stated that, in Figure 4, the absolute frequency of recombination is is plotted as a function of "the amount of 100% sequence homology in the first and second DNA sequences" of the positive-negative selector ("PNS") vectors. First of all, the x-axis of Figure 4 is labeled HOMOLOGY (KB) from 0 to about 14 KB. It is the number of kilobases of Hprt sequence; and it is not a measure of nucleotide sequence identity.

Then, the '764 Patent (column 21, lines 13-14) refers to Capecchi, M. R. (1989) Science 244: 1288-1292."("Capecchi 1989"), wherein Figure 3 of Capecchi 1989 titled "The targeting frequency at the hprt locus as a function of the extent of homology between targeting vector and the endogenous target" appears identical to the Figure 4 of the '764 patent. As discussed above, the term "extent of homology" refers to the **length** of endogenous and targeting hprt sequences which are of mouse origin. Capecchi 1989 in turn refers to Thomas 1987 for further details regarding the inactivation of the Hprt gene (page 1289, first column, fourth paragraph, first sentence; and second col. first full paragraph, second sentence). The Examiner alleges in the Office Action (page 18, lines 5-7) that it is unclear where in Capecchi 1989 does it refers back to Thomas 1987. Applicants point out that reference #8 mentioned at the end of those two sentences on page 1289 is Thomas 1987.

As discussed extensively above, the target Hprt DNA in Thomas is derived from

C57Bl/6 mouse ES cells, whereas the targeting Hprt DNA is obtained from the mouse ARK line (Thomas, page 511, under Experimental Procedures, left column, second paragraph, first sentence: "Hprt sequences were isolated from . . . a mouse ARK cell line"; and fifth paragraph, first sentence: "ES cells were isolated from C57Bl/6 blastocysts . . . ."). Thus, Figure 4 of the '764 patent is showing data based on recombination experiments wherein the targeting DNA and target DNA are clearly obtained from two **different** strains of mice. Accordingly, the "amount" as used in the brief description of Figure 4 of the '764 patent refers to the "number of kilobases", and the "100% sequence homology" refers to the relationship between the Hprt sequences in the PNS vector (targeting DNA) and in the ES cell (target DNA).

As mentioned in Applicants' Submission, one of skill in the art would recognize that it is not likely that the 14 Kb of genomic sequences in the two different strains of mouse used in the experiments reported in Thomas are base-by-base 100% identical[1]. There is no evidence to suggest that the entire 14 Kb Hprt sequences from the two different mouse strains had been sequenced, compared, and shown to be 100% identical. Therefore, Applicants contend that the inventors, Capecchi and Thomas, **would not** have used the term "100% sequence homology" in the brief description of Figure 4 and in the specification of the '764 Patent to describe targeting DNA and target DNA that are not known and not likely to be 100% identical.

Rather, the '764 Patent uses the term "100% sequence homology" to describe an evolutionary relationship (the precise meaning of the term according to Recce), i.e., target DNA and targeting DNA that are DNA sequences of the same gene from the same species of animal (e.g., mouse). This is the only consistent interpretation of the term permitted in the '764 Patent specification and his supporting data in the working example. One of skill in the art would have interpreted the '764 Patent as simply teaching the use of targeting and target DNA from the same species of animal.

Applicants address the Examiner's specific allegations in the Office Action as follows:

The Examiner alleges on page 15, lines 12-16 of the Office Action that "it appears that the first and second DNA sequences in the PNS vector are exactly the same as the corresponding sequences in the target DNA." The Examiner then refers to the '764 Patent, column 13, lines 37-42, wherein it is stated that Figure 3 of the '764 Patent depicts the structure of PNS vector comprising a first DNA sequence (exon I and a portion of a contiguous intron in the target DNA) and a second DNA sequence (adjacent portion of the same intron and exon

[1] To illustrate this point, Applicants refer to page 30 of the specification and Figure 2 of the instant application which analyzed the sequence divergence between the 129 strain and another BALB/c strain in the Rb locus. Numerous differences were identified in the 10.5 Kb region analyzed.

II). Applicants submit that one of ordinary skill in the art would understand that the passage and Figure 3 in the '764 patent teach that the positive selection marker be placed in a non-coding region (the intron between exon I and II of a target gene) such that upon recombination, the marker is located in the intron positioned between exon I and II of the same target gene. There is nothing in the passage to suggest that the first and second DNA sequences in the PNS vector must be exactly the same base-by-base as the corresponding gene sequences in the target DNA. Figure 3 actually shows one difference between the nucleotide sequence of the first DNA sequence in the PNS vector and the target DNA.

On page 17, lines 7-12, the Examiner alleges that, because the '764 Patent teaches that "substantial homology is necessary between portions in the PNS vector and the target DNA to insure targeting of the PNS vector to the appropriate region of the genome.", it follows that the skilled person would understand that the higher the homology the better the homologous recombination, and hence the phrase "100% sequence homology implies 100% sequence identity". Applicants respectfully point out that the sentence quoted by the Examiner discuss the accuracy of the targeting to an appropriate region of the genome, i.e., the location where homologous recombination would take place; and **not** the higher frequency of homologous recombination with a target gene. Targeting to the appropriate region of the genome is an all-or-nothing outcome which is categorically different from the Examiner's quantitative result of a "better" homologous recombination. Thus, contrary to the Examiner's contention, the skilled person would not conclude from the disclosure in the '764 Patent that the higher the homology the better (i.e., higher frequency) the homologous recombination. The '764 Patent does not support the contention that "100% sequence homology implies 100% sequence identity".

On page 18, lines 8-9 of the Office Action, the Examiner refers to an explanation for Figure 3 in Capecchi 1989, at page 1289, right column, first full paragraph, lines 8-10: "(the word "homology" is used here to describe participants in homologous recombination, which are generally identical)". Applicants submit that the term "identical" is modified by the adverb "generally" and one of ordinary skill in the art would understand that the flanking regions in the vector and the target DNA in the experiments described in Thomas (i.e., reference 8) are homologous and not 100% base-by-base identical. As discussed above and on pages 12-13 of the Submission, one of ordinary skill in the art would recognize that the 14 Kb of genomic sequences in each of the two different strains of mouse used in Thomas (reference in Capecchi Figure 3, and the '784 Figure 4) had not been sequenced and compared, and shown to be 100% identical.

The Examiner further alleges on page 18, lines 9-13 of the Office Action that, FIG 4 of the '764 patent shows that over the range tested, from 2.9 to 14.3 kb, a fivefold increase in

DNA sequence homology resulted in roughly a 100-fold increase in the targeting frequency. The Examiner surmises again that the higher the homology **or identity** the sequences have, the higher the targeting frequency would be. Here, the Examiner is equating homology with *identity*, which is untenable as discussed above. Applicants submit that the sentence is self-explanatory with respect to the meaning of "sequence homology", i.e., the fivefold increase in DNA sequence homology is the increase from 2.9 kb to 14.3 kb. Moreover, if the term "homology" is read as "identity" or even "similarity" as suggested by the Examiner, one of ordinary skill in the art would not understand how the sequence identity (or similarity) can be increased fivefold using the DNA from mice as described in the experiments.

Furthermore, when commenting on the neo^r gene that was transeferred, Capecchi 1989 states: "[i]n the above experiments, the amount of nonhomology (neo) being transferred to the target was kept constant." (page 1289, right column, first paragraph, line 13). The term "amount" and the phrase "being kept constant" both applies to the "nonhomology", i.e., the *length* of sequence encoding neomycin resistance. Still further, with respect to the neo^r gene that is transferred, it is referred to as "nonhomology". The neo^r gene, which originated from bacteria, possesses a different evolutionary origin from the mouse target DNA. Thus, Capecchi 1989 uses the term "homology" properly to indicate a common evolutionary origin as endorsed in Reeck.

In view of the foregoing, Applicants request withdrawal of the rejection based on the '764 Patent.

C. <u>The Rejection Based on the '764 Patent and the '260 Patent Is In Error</u>

Claims 89, 90 and 121 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over the '764 Patent in view of U.S. Patent No. 5,416,260 issued to Koller et al ("the '260 Patent"). The Examiner contends that the '764 Patent does not teach using cells from inbred 129 strain or BALB/c strain and that the '260 Patent teaches the use of mouse ES cell line E14TG2a, which was isolated from strain 129/01a embryos as target cells for homologous recombination. Applicants respectfully disagree with the rejection.

According to MPEP 2141.02, "[A] patentable invention may lie in the discovery of the source of a problem even though the remedy may be obvious once the source of the problem is identified. This is part of the 'subject matter as a whole' which should always be considered in determining the obviousness of an invention under 35 U.S.C. § 103." *In re Sponnoble*, 405 F.2d 578, 585, 160 USPQ 237, 243 (CCPA 1969). Here, the invention teaches that a relatively minor increase in DNA sequence similarity between target DNA and targeting DNA (as accomplished by using the same inbred strain as the sources) can effect an unexpected and

dramatic increase in efficiency of homologous recombination between target DNA and targeting DNA. The teachings of the prior art cited by the Examiner extend only as far as using DNA from the same animal **species**, and nothing more (see detailed discussions below).

According to *KSR Intern. Co. v. Teleflex, Inc.*, 127 S.Ct. 1727 (2007) at 1740, when the question is whether a patent claiming the combination of elements of prior art is obvious, . . . and if a technique has been used to improve one device, . . . a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions. In the present case, there is no hint or suggestion in the references that an improvement might be achieved through the use of target DNA and targeting DNA from the same inbred strain. It was indeed unpredictable at the time that the frequency obtained by practicing the claimed invention can be accomplished. Applicants have shown that using targeting construct and embryonic stem cells obtained from the same strain of mice, gene targeting was unexpectedly about 45-fold more efficient when compared to using targeting construct obtained from a different strain (specification at page 29, lines 25-37). Using targeting DNA of the claimed invention, about 75% of cells were correctly targeted without having to employ special selection techniques (specification at page 36, lines 9-15).

Without the present invention, one of ordinary skill in the art would continue to use targeting DNA and target DNA from different strains of mouse, and try to increase recombination frequency by maximizing the extent of the overlapping homologous sequences. The present invention, however, demonstrates for the first time the criticality of using target DNA and targeting DNA from the same inbred strain, thus identifying the source of the problem of low frequency of homologous recombination. That the claimed invention unexpectedly solved longstanding problems supported the conclusion of nonobviousness. *Hybritech Inc. v. Monoclonal Antibodies, Inc.* 802 F.ed 1367, 231 USPQ 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). Among the factors to be considered in determining whether a claimed invention is obvious are objective indicia of nonobviousness, such as commercial success of the invention, the satisfaction of a long-felt but unmet need, failure of others, and unexpected results. *See Graham*, 383 U.S. at 17-18, 148 U.S.P.Q. at 467; *Minnesota Mining & Manufacturing Co. v. Johnson & Johnson Orthopedics, Inc.*, 976 F.2d 1559 at 1573, 24 U.S.P.Q. 2d 1321 at 1333. As discussed *supra*, in the Submission and the Declaration, only after disclosure by the inventors of the present invention, for example, in Riele, did the practice of the scientists in the field started to change. This shows that the present invention satisfies a long-felt but unmet need.

Accordingly, the claimed invention is non-obvious over the prior art references, alone or combined.

In particular, Applicants submit that the '764 Patent and the '260 Patent merely disclose various genetic constructs for use in gene targeting by homologous recombination in embryonic stem cells. Specifically, the '764 Patent teaches the use of positive-negative selector ("PNS") vectors constructed from a plasmid pAT-153 (the '764 Patent, col. 22, lines 49-51) that contained mouse DNA isolated from the BALB/c strain of mouse.[2] The '764 Patent teaches that the PNS vectors are introduced into ES cells that are derived from either C57 Bl/6 or CC1.2 (col. 23, lines 40-66). Evidently, the targeting DNA and the target DNA used in the '764 Patent were from different mouse strains.

The '260 patent teaches using ES cells that are derived from E14TG2a which was isolated from strain 129/01a embryos (col. 10, line 65; col. 13, lines 15-16; col. 14, lines 35-36). The targeting plasmid pKCβ_2B was described as containing the entire β_2-microglobulin gene within an 8.4 kb Xho I fragment (col. 10, lines 38-41) that was obtained from a C57BL6/N genomic DNA library[3]. Thus, the β_2-microglobulin gene DNA in the targeting plasmid is obtained from a mouse strain different from the target β_2-microglobulin gene in the ES cells.

Neither references, either alone or in combination, teach or suggest using the same mouse strain for both targeting DNA and target DNA in homologous recombination. Neither teaches or suggests the criticality or benefit of using DNA from the same inbred mouse strain for the targeting DNA and target DNA in homologous recombination. Applicants suggest that the Examiner has perhaps unwittingly used hindsight to arrive at the present invention. Such hindsight reconstruction, however, is improper.

Accordingly, the '764 Patent in view of the '260 Patent do not render claims 89, 90 and 121 obvious. Applicants request withdrawal of the rejection based on the '764 Patent in view of the '260 Patent.

D. The Rejection Based on Capecchi C06 or the '764 Patent and Doetschman Is In Error

Claims 89-98 and 100-127 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Capecchi C06 or the '764 Patent each in view of Doetschman et al., 1987, Nature 330:576-578 ("Doetschman"). The Examiner alleges that it would have been prima facie obvious to use flanking sequences in a targeting DNA construct that are 100% identical to

---

[2] The plasmid pAT-153 is described in Peters et al., 1983, Cell, 33:369, submitted herewith as Reference C78 . See, page 369, right column, 4th paragraph, lines 1-2 under "Cloning of Virus-Cell Junctions".

[3] The '260 patent refers to Ozato and Orrison (1985) and Warner et al. (1987) regarding the β_2-microglobulin gene, both submitted herewith as Reference C77 and C80, respectively. However, there was no mention of the cloned β_2-microglobulin gene in these two references. Applicants identified Margulies et al. (Proc Natl Acad Sci U S A. 1983 April; 80(8): 2328–2331, a copy submitted as Reference C75) which discloses a 8.4 kb Xbo I fragment containing the β_2-microglobulin gene cloned in the plasmid vector pKC7 (see page 2328, col. 2, under "Genomic Clones", lines 1-5).

the corresponding sequences in the target locus of ES cells. The Examiner points to the following in Doetschman (p. 577, left column, lines 13-15 and 21-22, and Fig. 1):

> Homologous recombination between a target chromosomal locus and exogenous DNA having sequences *in common* with the target[7-14] has this potential, ...The correcting plasmid, pNMR133, has between 2.5 and 5kb of DNA *in common* with the target locus (more precise definition of the length awaits exact determination of the exact endpoint of the deletion).(emphasis added)

The Examiner is asserting that the phrase "has between 2.5 and 5kb of DNA in common with the target locus" is a suggestion that the flanking sequences in the plasmid (2.5 and 5kb) should be 100% identical to the corresponding sequences in the target locus. However, there is nothing in Doetschman that suggests that "2.5 and 5kb of DNA in common with the target locus" mean 100% identical to the corresponding sequences in the target locus.

First, Applicants point out that the 2.5 and 5 kb of the DNA in the correcting plasmid, pNMR133, were obtained from a BALB/c mouse HPRT gene (Doetschman, p.577, Fig. 1 legend, line 2, and reference 18). In reference 18, Melton et al. (1984, PNAS 81:2147-2151; "Melton", submitted herewith as Reference C76) used DNA from a BALB/c mouse to construct a genomic DNA library to clone the HPRT gene (Melton, page 2147, right column, lines 2-3). Doetschman used the same ES cell line, E14TG2a, as discussed above. Applicants emphasize that DNA from different mouse strains i.e., BALB/c HPRT genomic DNA in the vector and HPRT gene in the ES cells prepared from 129/01a embryos, were used in Doetschman's experiments.

Applicants point out that, Doetschman admits in the same sentence that it has limited knowledge about the length of the DNA that is in common. It is unlikely that the exact nucleotide sequences of the **common** DNA regions in the vector and in the ES cells were known. Apparently, Doetschman was satisfied that the Hprt gene sequences are both of mouse origin, and that there are about 2.5 kb and 5 kb of flanking sequences.

Moroever, Doetschman removed a HindIII site in one of the **common** regions by the insertion of 4 bp during construction of the plasmid (page 577, Figure 1 legend, lines 13-14, site of deleted HindIII is marked as by (-) in the plasmid; (+) indicates original HindIII site in the gene). Thus, one of the flanking regions that are in **common** are not 100% identical. This modification of the HPRT gene in the plasmid shows that Doetschman was not concerned with 100% sequence identity between the common regions in the targeting plasmid and the target locus. Applicants submit that the design of the targeting plasmid in Doetschman teaches

against the Examiner's contention that the combined teachings of Doetschman and Capecchi C06 or the '764 Patent suggest the use of flanking sequences in the targeting DNA that are 100% identical to the corresponding sequences in the genome of the target cells.

One of ordinary skill in the art would have understood the term "**common**" as used in Doetschman to mean targeting and target DNA with a **common evolutionary origin**, i.e., from the same species of animal. Since the Examiner admits that Capecchi C06 and the '764 Patent does not teach using flanking sequences in the targeting DNA construct that are 100% identical to the corresponding sequences in the genome of target cells, and Doetschman does not teach or suggest using the same mouse strain for targeting DNA and target DNA in homologous recombination, none of the reference, either alone or in combination, teach or suggest using the same mouse strain for targeting DNA and target DNA in homologous recombination. Furthermore, as discussed above, none of Capecchi C06, the '764 Patent and Doetschman teach the criticality or benefit of using the same mouse strain for targeting DNA and target DNA in homologous recombination. Accordingly, Capecchi C06 and the '764 Patent each in view of Doetschman do not render claims 89-98 and 100-127 obvious.

Applicants request withdrawal of the rejection based on Capecchi C06 and the '764 Patent each in view of Doetschman.

E. Rejection Based on Nonstatutory Obviousness-Type Double Patenting

Claims 89-98, 100-127 have been rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-23 of U.S. Patent No. 6,653,113 to Berns ("the '113 patent") and claims 1-18 of U.S. Patent No. 5,789,215 ("the '215 patent") to Berns. In response, while not admitting that the claims of the above-identified patent application are not patentably distinct from claims 1-23 of the '113 patent or claims 1-18 of the '215 patent, Applicants, upon indication of allowable subject matter, will submit a Terminal Disclaimer under 37 C.F.R. § 1.321(c) for the above-identified application.

CONCLUSION

Entry of the foregoing amendments and consideration of the foregoing remarks are respectfully requested. No fee is believed to be due for this amendment. Should any fee be required, please charge such fee to Jones Day Deposit Account No. 50-3013. Applicants respectfully submit that all claims are now in condition for allowance. The Examiner is invited to call the undersigned attorney if a telephone call could help resolve any remaining issues.

Respectfully submitted,

Date: May 19, 2008

Laura A. Coruzzi — 30,742 (Reg. No.)

By: T. Christopher Tsang — 40,258 (Reg. No.)

Jones Day
222 East 41st Street
New York, N.Y. 10017
(212)-326-3939